Development from Egg to Juvenile of the Red Grouper *(Epinephelus morio)* (Pisces: Serranidae) in the Laboratory

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Abstract

Red groupers were reared from artificially spawned eggs, which hatched in 30 hours at 24°C. Eggs required salinities of at least 32 ppt to float. Yolk absorption was completed by about 30 hours, eye pigmentation and first feeding observed at 72 hours post-hatch. Dorsal and pelvic spine development was first noted at nine days post-hatch with rapid length increase over the next seven days. Flexion occurred starting at 16 days post-hatch at a stage with well developed dorsal and pelvic spines. Behavior of larvae was closely examined and at all stages larvae were active predators on small zooplankton. Metamorphosis occurred at a minimum of 35 days post-hatch at 20 mm SL. Juveniles grew at a rate between 0.26 and 0.61 mm·day^{-1} reaching a mean of about 55 mm SL at 130 days post-hatch and were aggressive and territorial. Reared juveniles tagged and released on an artificial reef off the Florida Panhandle survived and continue to be monitored.

Resumen

El mero americano fué criado de huevos desovados artificialmente. Los huevos requirieron salinidades de al menos 32 ppt para flotar y fueron incubados durante 30 horas a 24°C. La absorción del saco vitelino se completó a las 30 horas, la pigmentación y primera alimentación se observó a las 72 horas después de la incubación. El desarrollo de las espinas dorsal y pélvica fué observado primero a los 9 días después de la incubación con un rápido incremento en longitud durante los 7 días siguientes. La flexión ocurrió al inicio, 16 días después de la incubación con las espinas dorsal y pélvica bien desarrollada. El comportamiento de las larvas fué examinado estrechamente y durante todos los estados larvarios fueron predadores activos sobre pequeño zooplancton. La metamorfosis ocurrió a un mínimo de 35 días después de la incubación a 20 mm de longitud estandar (SL). Los juveniles crecieron a una tasa entre

0.26-0.61 mm por día, alcanzando un promedio de 55 mm SL a 130 días después de la incubación; y fueron agresivos y territoriales. Los juveniles criados fueron marcados y liberados en un arrecife artificial en aguas fuera de Florida donde los supervivientes continúan siendo monitoreados.

Introduction

The red grouper (*Epinephelus morio*) is the most common grouper captured in both commercial and recreational fisheries in US and Mexican waters of the Gulf of Mexico (Goodyear and Schirripa 1991). In the Exclusive Economic Zone of the US Gulf of Mexico, it accounts for two-thirds of the total commercial grouper catch, some 3 400 t in 1989 (Goodyear and Schirripa 1991). Despite its commercial and recreational importance, relatively little has been published on its biology since the study of Moe (1969) (see Brulé and Déniel, this vol.).

Although the larvae of epinepheline serranids have been recognized for more than a century (Kendall 1984), largely due to their elongate dorsal and pelvic fin spines, few identifiable to a particular species have been described in detail. Virtually nothing is known of the specific larval development of *E. morio* (Moe 1969) and the wild-captured larvae could not be separated from *E. guttatus* and *E. drummondhayi* based on ray counts (Johnson and Keener 1984).

As part of a study to delineate life histories of Gulf of Mexico groupers, we were able to rear red groupers in the laboratory from artificially fertilized eggs through the juvenile stage. These laboratory-reared juveniles were released in the field successfully and have survived.

Materials and Methods

Live adult *E. morio* females were obtained by fishing from commercial hook-and-line fishing boats during the spawning season in the northern Gulf of Mexico. Fish captured at depths between 20 and 40 m had their swim bladders deflated immediately upon surfacing and were placed in flowing seawater tanks. Survival rates were high unless the fish had been gill-hooked or otherwise injured. Individual fish were returned to the Florida State University Marine Laboratory and maintained in 2 000-l tanks in a closed-circuit seawater system.

Freshly caught ripe *E. morio* males were obtained from commercial fishers at the dock. Testes were removed from the body cavity within a few hours of capture and stored in plastic bags on ice (not frozen). Sperm remained capable of fertilizing eggs for a number of days afterwards.

In the aquarium facility at Florida State University Marine Laboratory (FSU), live fish were catheterized to determine sex and stage of gonad development. Females were injected with 1 000 units human chorionic gonadotropin (HCG) per kg body weight, followed, if necessary, by a second injection of 500 units HCG 18-24 hours later.

Adult fish were maintained in recirculating seawater of approximately 25 ppt (the salinities of inshore areas of North Florida during spring) until eggs were fully hydrated. Once hydrated, eggs were stripped from females, into plastic containers, with gentle pressure on the body cavity. A sperm and water mixture was added immediately. Sperm were obtained either by stripping live male fish or macerating a piece of fresh or chilled testes. An additional few milliliters of sea water (36 ppt) were added to the sperm and egg mixture, which caused the eggs to become buoyant.

Initiation of development was confirmed microscopically by the onset of first cleavage and occurred within an hour of addition of sperm. Eggs were then transferred

to 20-l aerated buckets of 36 ppt seawater at 22°C, and then moved to the rearing aquariums (36 ppt seawater) within 3-4 hours.

The absolute density of eggs and newly hatched larvae was determined by pipetting eggs and larvae into small beakers with seawater of different salinities (between 27 and 35 ppt): eggs and larvae were either negatively buoyant, positively buoyant or neutrally buoyant.

Larvae were reared largely using the methods of Houde and Taniguchi (1977). Floating fertile eggs were transferred to 80-l aerated rearing aquariums with twin 20-W flourescent lights. Larvae were fed zooplankton collected offshore of the FSU Marine Laboratory starting approximately 72 hours after hatching (depending on water temperature). Zooplankton was maintained on various species of cultured microalgae, which also served to absorb toxins generated in the rearing tanks. Initially, plankton 53-88 µ in size were offered, followed a few days later by plankton in the 53-125 u size fraction.

Larvae were preserved every few days in order to obtain a representative size series for describing developmental stages. Preservation was initially in 5% formalin and later in 70% ethanol. The specimens selected for preservation at any one time were generally among the larger, more advanced individuals in the rearing tanks. Specimens were measured either with dial calipers or by ocular micrometer in a dissecting microscope. Preflexion larvae were measured from the tip of the snout to the end of the notochord (NL) whereas post-flexion larvae and juveniles were measured to standard length (SL). All development times given in this paper (e.g., 24 hours, 5 days) are times post-hatching.

Several hours of excellent video recordings of live larvae were obtained using a dissecting scope with attached color video camera. Larvae were chilled to make them quiescent. Additional recordings were made of larvae remaining in rearing tanks by using additional closeup diopters and extreme telephoto settings on the video camera lens. Drawings of larvae were made by one of us (WAL) using a camera lucida attachment on a stereo microscope. Color illustrations were prepared from fresh specimens.

The series of larval specimens are deposited in the US National Museum as lot number USNM 326839.

Results

Eggs were almost spherical, approximately 0.95 mm in diameter. They contained a single oil globule and were unpigmented. The germinal disk of the eggs was visible 25 minutes after addition of sperm; first cleavage occurred 54-59 minutes post-fertilization. Hatching occurred 30-38 hours post-fertilization, although the exact time of hatching was not observed.

Fertilized eggs and newly hatched red grouper larvae were positively buoyant in full-strength seawater (36 ppt); fertilized eggs were negatively buoyant in 25 ppt seawater. Eggs at 4 hours post-fertilization were neutrally buoyant in seawater between 28 and 30 ppt. Eggs at over 20 hours post-fertilization were slightly more dense; they were neutrally buoyant in 32 ppt. Newly hatched larvae were more positively buoyant at 27 ppt, with some floating and some sinking. Eggs in 25 ppt seawater underwent limited embryonic development while on the bottom of the container. Very few of these eggs hatched. None of the larvae that hatched at this salinity was reared.

Larval and pelagic juvenile development

Meristics and morphometrics. Meristic and morphometric data on larvae and juveniles were based on a series of 44 specimens (Tables 1 and 2). The larval and early

juvenile stages are illustrated in Figs. 1 to 10. Members of the genus *Epinephelus* have a single dorsal fin; thus, all subsequent references to the second dorsal fin spine refer to the second spine of the dorsal fin, not to a second of two dorsal fins.

Development of yolk sac larvae. Yolk sac larvae up to about 48 hours (Fig. 1) hatch at 1.6 to 1.7 mm NL. At this stage they hang head down in the water column. The yolk and oil globules were absorbed by 3 days (Fig. 2, lower). Eye pigmentation is first evident at about 60 hours; eyes are darkly pigmented by about 72 hours. At this stage the larvae drift in the water column, not orienting to gravity or light and react to pressure waves by an undirected burst of swimming for a few seconds.

Shortly after 3 days, the larvae begin maintaining orientation in the water column. The mouth has been formed and the gut is functional. First feeding is initiated a few hours post-mouth formation and is evidenced by feeding strikes at plankters.

Table 1. Meristics of larval and juvenile *Epinephelus morio*, based on unstained specimens. Counts of pelvic fin elements (I,5) and superior and inferior principal caudal fin rays (8,7) were constant throughout the series following development. [*Relaciones merísticas de larvas y juveniles de* Epinephelus morio *basado en especímenes no teñidos. El conteo de los elementos de la aleta pélvica (I, 5) y los rayos de la aleta caudal principal superior e inferior (8,7) fueron constantes a lo largo de las series siguiendo el desarrollo.*]

Age	Stage	Standard length	Dorsal fin spines and rays	Anal fin spines and rays	Pectoral fin rays
3	Preflexion	2.5	-	-	-
4	Preflexion	2.5	-	-	-
4	Preflexion	2.6	-	-	-
4	Preflexion	2.6	-	-	-
6	Preflexion	2.6	-	-	-
8	Preflexion	2.8	-	-	-
8	Preflexion	2.8	-	-	-
9	Preflexion	2.9	I	I	-
9	Preflexion	3.3	I	-	-
9	Preflexion	3.5	I	-	-
11	Preflexion	3.7	II	-	-
11	Preflexion	3.7	II	-	-
11	Preflexion	3.8	II	-	-
12	Flexion	4.1	III	-	-
15	Flexion	5.5	III	-	-
18	Flexion	5.7	III	-	-
16	Flexion	6.2	IV, forming	forming	-
20	Flexion	6.6	IV, forming	forming	forming
17	Flexion	7.0	V, 6	I,8	forming
18	Flexion	7.4	IV, forming	forming	forming
20	Flexion	7.4	XI, 16	II+1,9	forming
21	Postflexion	8.8	X+1, 16	II+1,9	17
21	Postflexion	9.3	X+1, 16	II+1,9	17
21	Postflexion	9.5	X+1, 15	II+1,9	16
21	Postflexion	9.6	X+1, 15	II+1,9	16
27	Transforming	12.2	X+1, 15	II+1,9	16
27	Transforming	15.1	X+1, 14	II+1,9	16
25	Transforming	20.2	XI, 15	III,9	17
25	Transforming	26.2	XI, 16	III,9	17

Table 2. Body proportion of larvae and juveniles of *Epinephelus morio*. Values given are percent of standard length (SL) and head length (HL) including mean, standard deviation and range in parentheses. Number of specimens measured indicated in parentheses, listed by stage. [*Proporción corporal de larvas y juveniles de* Epinephelus morio. *Los valores dados son porcentajes de la longitud estandar (SL) y longitud de la cabeza (HL) incluyendo media, desviación estándar e intervalo entre paréntesis. El número de especímenes medidos es indicado entre paréntesis, listado por estado.*]

	Body depth at cleithrum (SL)		Body depth at anus (SL)		Caudal peduncle depth (SL)	
Yolk sac (15)	30.5±9.3	(18.4-45.3)	8.8±1.7	(6.6-12.7)	-	-
Preflexion (13)	19.9±1.8	(17.5-24.2)	8.6±1.5	(6.7-11.6)	-	-
Flexion (8)	28.2±3.1	(22.9-31.8)	17.2±3.8	(9.8-22.6)	8.8±0.5	(8.4-9.6)
Postflexion (4)	32.4±0.9	(31.2-33.6)	24.5±0.3	(24.1-24.9)	11.4±0.3	(11.1-11.9)
Transforming (2)	31.8+1.4	(30.4-33.2)	24.8±0.8	(24.0-25.7)	11.7±0.5	(11.3-12.2)
Juvenile (2)	34.6±1.5	(33.1-36.1)	27.5±0.8	(26.7-28.2)	11.5±0.0	(11.4-11.5)
	Head length/ SL		Eye diameter/ Head length		Upper jaw length/, Head length	
Yolk sac (15)	19.9±3.2	(16.0-27.0)	50.8±10.5	(40.0-77.8)	-	-
Preflexion (13)	24.8±3.3	(18.8-30.0)	42.3±5.1	(37.1-53.3)	38.7±7.0	(26.3-50.0)
Flexion (8)	34.6±2.5	(30.3-37.7)	31.9±2.1	(28.6-35.0)	41.0±4.0	(34.1-46.4)
Postflexion (4)	37.6±0.8	(29.7-32.1)	30.6±1.0	(29.7-32.1)	42.9±4.2	(38.1-49.5)
Transforming(2)	37.7±2.0	(35.7-39.6)	28.2±0.6	(27.6-28.8)	47.9±0.2	(47.7-48.1)
Juvenile (2)	41.3±1.0	(23.8-25.7)	24.8±1.0	(23.8-25.7)	41.1±2.0	(41.1-45.0)
	Snout length/ Head length		Longest anal spine length/SL		Pectoral fin length/SL	
Yolk sac (15)	-	-	-	-	-	-
Preflexion (13)	24.1±0.8	(22.9-25.0)	-	-	8.5±2.3	(4.8-11.6)
Flexion (8)	28.6±4.3	(19.6-33.3)	7.6±2.2	(5.2-10.9)	9.6±0.9	(8.9-11.4)
Postflexion (4)	31.1±2.2	(28.8-34.6)	11.7±0.2	(11.7-12.1)	13.1±1.0	(12.0-14.7)
Transforming(2)	25.1±0.8	(24.4-25.9)	12.6±1.3	(11.3-14.0)	17.0±0.3	(16.6-17.3)
Juvenile (2)	25.1±1.7	(23.4-26.8)	17.8±0.6	(17.2-18.4)	26.6±0.7	(25.6-27.7)

Fin formation. The sequence of fin development is pectoral, spinous dorsal and spinous pelvic simultaneously, caudal, soft dorsal and soft anal simultaneously, and finally the spinous anal. Elements of the caudal, the soft dorsal and anal, and spinous anal develop from the center of each fin outwards in both directions. Development begins anteriorly and progresses posteriorly in the spinous-dorsal-fin elements; it progresses from dorsal to ventral in the pectoral fin.

Pectoral fin development begins in larvae 2.6 mm NL. Pectoral fin length increases from 5.4 to 25.6% of NL or SL between preflexion and transformation stages. Fin elements begin to develop at 6.6 mm, attaining the full adult complement of fin rays by 9.5 mm.

Dorsal and pelvic fin spines begin to develop simultaneously at 7 days (Fig. 2, center). The anlagen of the second dorsal spine first appears at 2.8 mm, initially visible only as nubs, protruding from the myomeres into the finfold, but subsequently protrude beyond the fin fold at 8-9 days (Fig. 2, upper). The second dorsal spine and pelvic spine are the longest elements in their respective fins throughout the larval development.

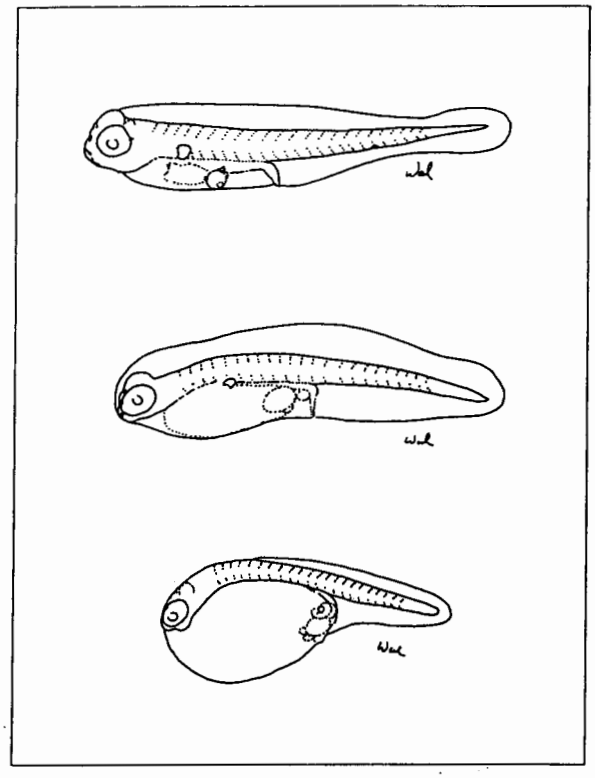

Fig. 1. Yolk sac larvae of *Epinephelus morio*, laboratory-reared. Upper: At hatch, 1.7 mm NL; Middle: 24 hours, 2.3 mm NL; Lower: 48 hours, 2.4 mm NL. [*Larva de* Epinephelus morio *con saco vitelino, criado en laboratorio. Arriba: Al incubarse 1.7 mm NL; en el medio, a las 24 horas, 2.3 mm NL; Abajo: a las 48 horas con 2.4 mm NL.*]

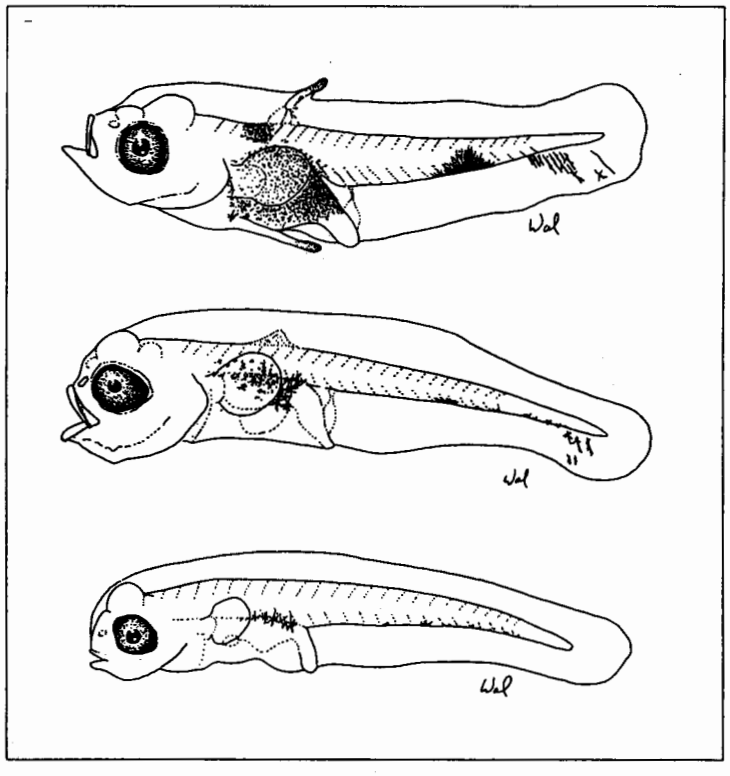

Fig. 2. Early stage laboratory-reared larvae of *Epinephelus morio*. Lower: 3 days, 2.7 mm NL, at time of first feeding; Middle: 8 days, 3.0 mm NL, with dorsal fin spine bud visible; Upper: 9 days, 3.5 mm NL, developing second dorsal fin and pelvic fin spines; the ventral melanophore spot is well-developed. [*Estadio temprano de larva de* Epinephelus morio *criadas en laboratorio. Abajo; 3 días con 2.7 mm. NL, al tiempo de primera alimentación; En el medio, 8 días con 3.0 mm NL, con el brote de la espina de la aleta dorsal visible. Arriba: 9 días con 3.5 mm NL, con las espinas de la segunda aleta dorsal en desarrollo, el melanóforo ventral está bien desarrollado.*]

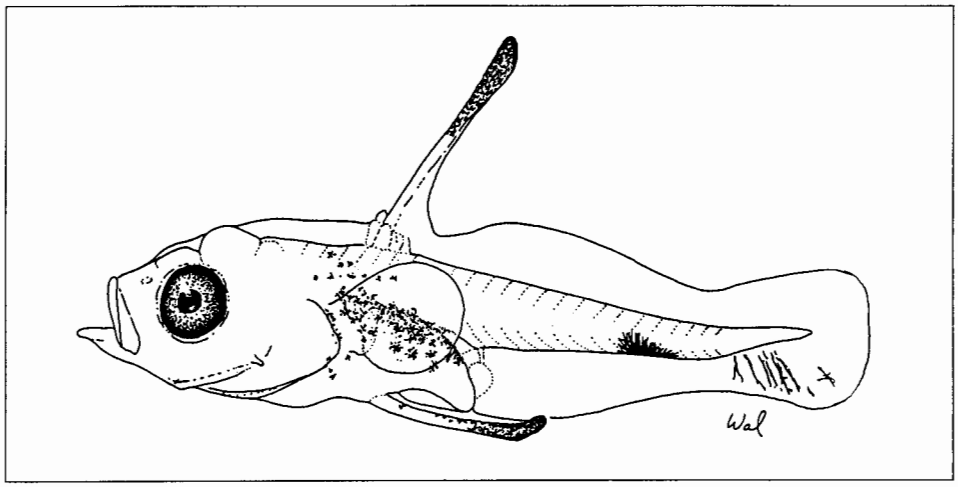

Fig. 3. Twelve-day larvae of *Epinephelus morio*, 4.1 mm NL. Initial ossification of the caudal fin supporting elements can be seen. Dark ends of the dorsal and pelvic fin spines are evident. [*Larvas de 12 días de* Epinephelus morio, *4.1 mm NL. Puede observarse una osificación inicial de la aleta caudal soportando los elementos. Los extremos opacos de las espinas de las aletas dorsal y pélvicas son evidentes.*]

Development of the second dorsal and pelvic fin spine is extremely rapid over the next 7-10 days (Figs. 3 and 4). For larvae 4-10 mm SL pelvic fin spine length was 72-88% of second dorsal fin spine length. The spines reach their longest length relative to SL when larvae are 5.5-7.5 mm SL (15-20 days post-hatch) (Figs. 4-6). Some of the largest specimens (>15 mm SL) had the pelvic fin spine somewhat longer than the second dorsal fin spine.

Development of the first dorsal spine is next, in larvae at about 3.7 to 3.8 mm SL (11 days) (Fig. 3). It develops a few small spinelets. It is followed by development of the third dorsal spine, forming at about 4.1 mm SL (12 days) (Fig 4).

Soft dorsal, soft anal, and caudal fin rays begin development at 5.5 mm. The adult complement of eight superior and seven inferior principal rays develops by

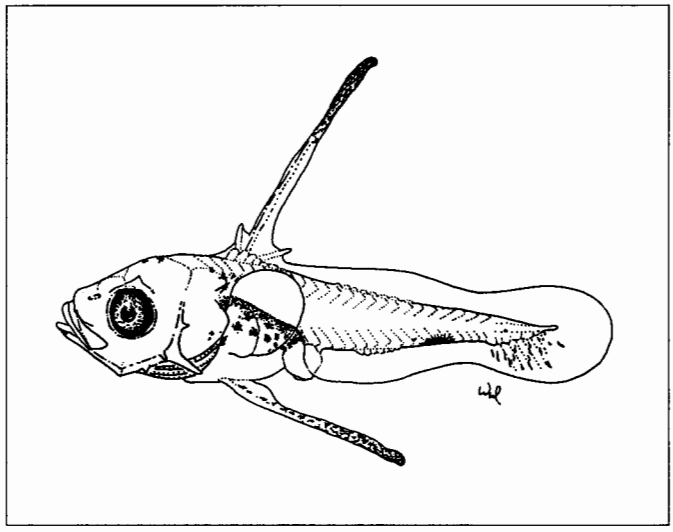

Fig. 4. Thirteen-day larvae of *Epinephelus morio*, 5.8 mm NL, showing further development of spination, including elongation of the second dorsal and pelvic fin spines, appearance of the first and third dorsal fin spines and appearance of the preopercular spines. This stage is at the greatest relative development of the elongate spines. Flexion is just beginning to occur. [*Larva de 13 días de* Epinephelus morio *de 5.8 mm NL mostrando el futuro desarrollo de las espinas, incluyendo el alargamiento de las espinas de la segunda aleta dorsal y de la aleta pélvica, aparición de las espinas de la primera y tercera aleta dorsal, y aparición de las espinas preoperculares. En este estado se observa el más grande desarrollo relativo del alargamiento de las espinas. La flexión está a punto de ocurrir.*]

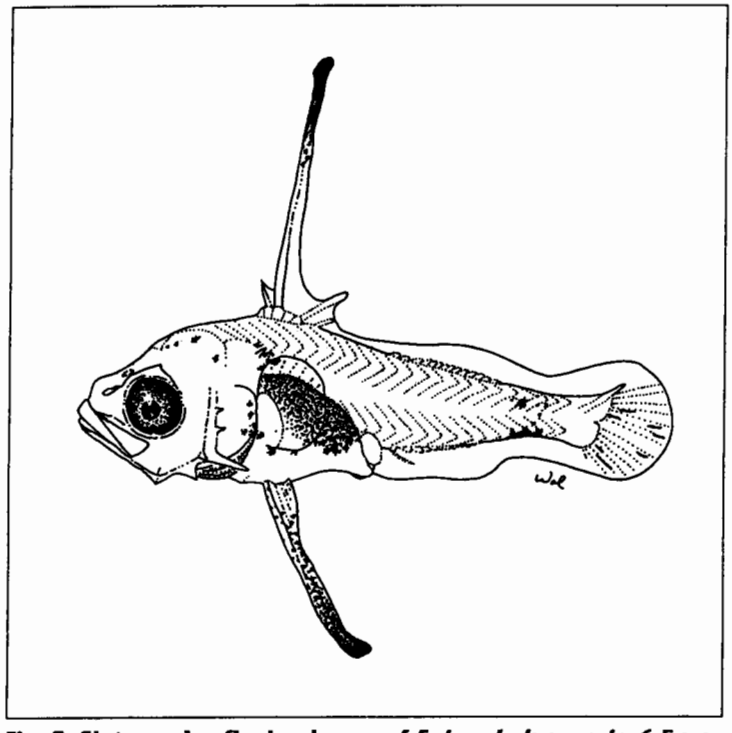

Fig. 5. Sixteen-day flexion larvae of *Epinephelus morio*, 6.5 mm NL. [*Larva flexionada de 16 días de* Epinephelus morio, *de 6.5 mm NL.*]

7.4 mm. The adult complement of soft dorsal (15 or 16) and anal fin (9) rays develops by 9.3 mm. The spinous anal fin is the last fin to begin development at 7.4 mm. The third anal fin spine is the last fin element to ossify at 20 mm.

The dorsal and pelvic spines are sheathed with tissue and have heavily pigmented fleshy lobes at their tips from the time of their first appearance through the juvenile stage (Figs. 3 to 10). The mobility of the dorsal and pelvic spines are described in Colin and Koenig (in press).

Spination of the head. Spines on the head begin to develop at 4 mm. The

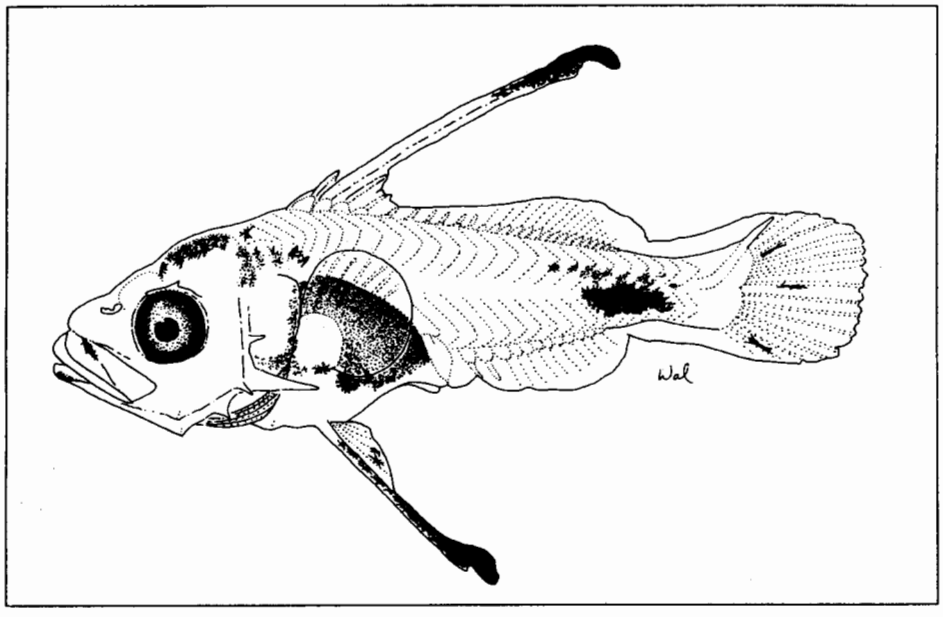

Fig. 6. Eighteen-day post-flexion larvae of *Epinephelus morio*, 7.4 mm SL. [*Larva de 18 días en etapa de post-flexión de* Epinephelus morio, *de 7.4 mm SL.*]

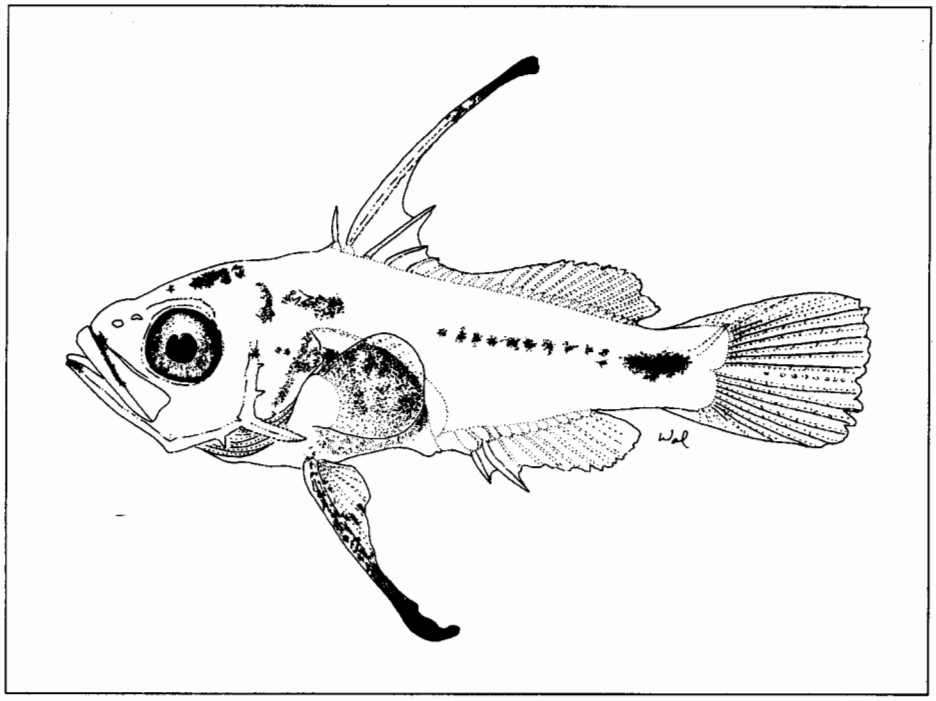

Fig. 7. Twenty-one-day post-flexion larvae of *Epinephelus morio*, 9.8 mm SL. [*Larva de 21 días en etapa de post-flexión de* Epinephelus morio, *de 9.8 mm SL.*]

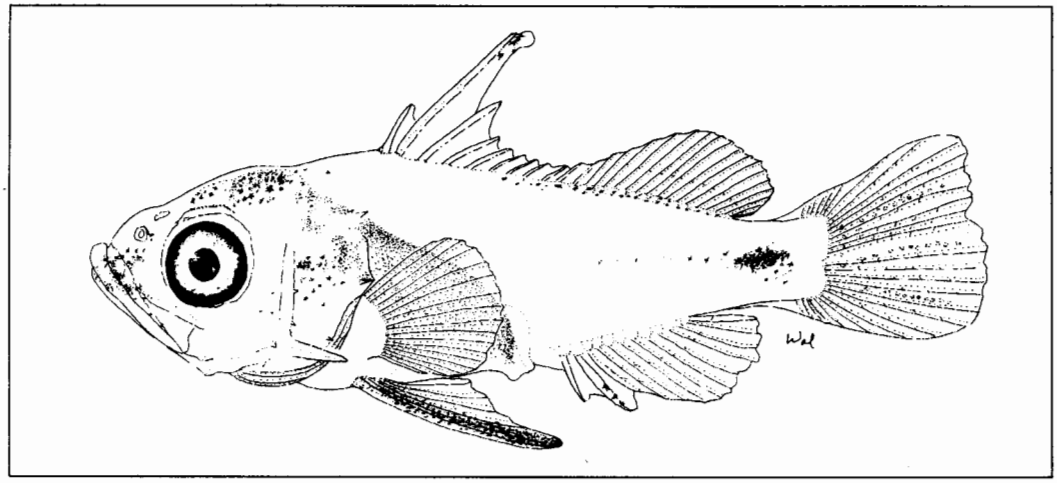

Fig. 8. Twenty-seven-day pelagic juvenile of *Epinephelus morio*, 12.6 mm SL. [*Juvenil pelágico de* Epinephelus morio *de 27 días, de 12.6 mm SL.*]

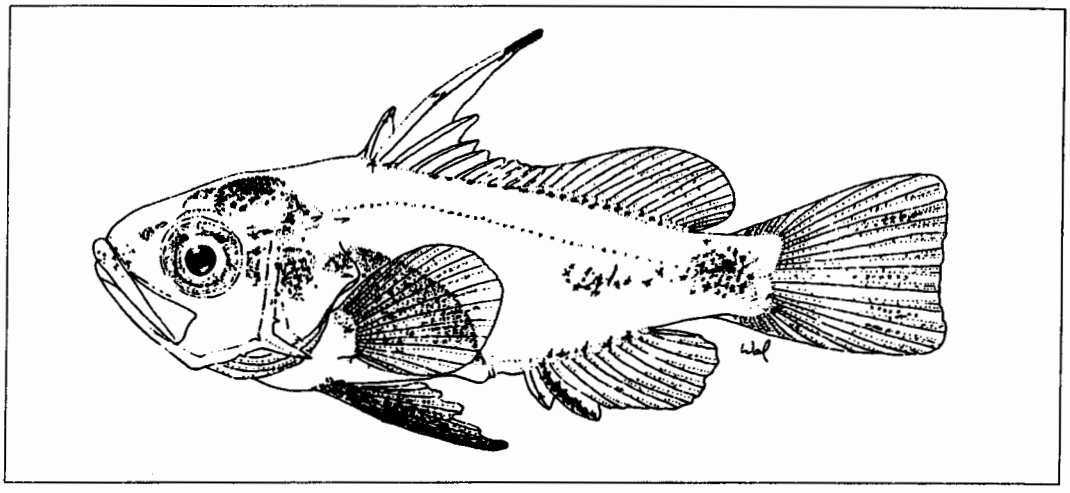

Fig. 9. Twenty-seven-day pelagic juvenile of *Epinephelus morio*, 13.9 mm SL. [*Juvenil pelágico de* Epinephelus morio *de 27 días, de 13.9 mm SL.*]

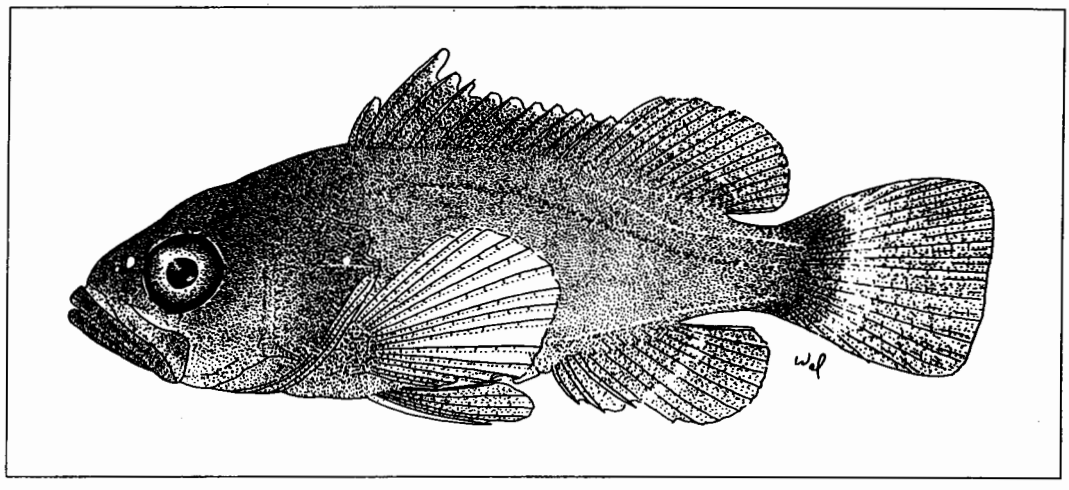

Fig. 10. Sixty-day laboratory-reared benthic juvenile *Epinephelus morio*. [*Juvenil bentónico de* Epinephelus morio *criado en laboratorio de 60 días.*]

angle spine of the posterior preopercular series forms first. Subsequent preopercular spines form from dorsal to ventral along the posterior margin away from the angle spine. Single spines develop on each side of the angle spine, at the angle of the anterior margin of the preopercle, on the frontal bone over the eye (supraocular spine), and on the supracleithral bone by 5.3 mm. Two or three preopercular spines appear dorsal to the angle spine and one spine remains ventral to the angle spine from post-flexion (6.8 mm) through transformation (20 mm). A second supracleithral spine develops by 7.4 mm. The single supraocular spine is reduced in size and becomes part of a serrated supraocular ridge on the frontal bone by 9.6 mm. A subopercular and an interopercular spine begin to develop by 9.6 mm. During the transformation stage

several spines become overgrown with tissue and/or bone: the spine ventral to the angle spine, the angle spine of the anterior peropercular margin, and the supracleithral spines. Posterior preopercular spines on the dorsal margin rapidly increase in number, with 12 small spines giving a serrated appearance in small juveniles.

Lateral line and scale formation. Lateral line organs (indicated by a row of light colored spots on the flesh and caudal fin membrane) are visible on transforming specimens (15.1 mm). Scale development also appears to begin on the dorsal surface of the body and proceed from anterior to posterior (15.1 mm). The body is fully-scaled by the beginning of the juvenile period (20 mm).

Pigmentation: general. Larvae lack pigmentation at hatching. Pigmentation is first observed on 2.4 mm larvae as two small melanophores on the tip of the snout, two small melanophores over the forebrain and three small melanophores at the anterior to the yolk sac. The eyes become pigmented by day 3, with a scattering of melanophores over the gut and along the ventral area of the tail.

As the larvae grows, the caudal pigment spot moves slightly to the posterior onto the caudal peduncle and dorsally to the level of the centra. When the elongate dorsal and pelvic spines are folded back their dark tips are located very close to the caudal spot. In live larvae, the caudal end of the body is nearly transparent and it is sometimes difficult to distinguish the caudal spot from the dark tips of the spines.

From the earliest stages of dorsal and pelvic fin spine formation, the spines have fleshy tips with dense melanophores. As these spines lengthen greatly, the dark markings on their tips become more prominent, covering the outer one-third to one-half, making the spines readily apparent (Colin and Koenig, in press). The tips of other spines of the dorsal fin and the anal fin are not pigmented in larvae. Late stage larvae acquire melanophores on the upper surface of the head and a line along the base of the dorsal fin.

Pigmentation: head region. Head pigmentation is lacking on most preflexion larvae (2.5-3.8 mm). Two preflexion larvae were observed with a melanophore located on each side of the ventral midline near the tip of the lower jaw while one specimen had only one of the two melanophores. This pair of melanophores near the tip of the lower jaw is present on all flexion larvae (5.5-7.4 mm). Flexion larvae >5 mm have a melanophore on the upper jaw on the anterior margin of the maxillary. Melanophores generally increase in number over the dorsal surface of the midbrain and forebrain during the flexion stage. By 7.4 mm, a cap of melanistic pigmentation covers the dorsal surface of the midbrain. In larvae >5 mm, internal melanophores appear over the hind brain and extend anteriorly from the anterior end of the notochord onto the perineural sheath in the nape region. Melanophores first appear on the opercle and color first appears on the head during post-flexion (9 mm).

Yellow (xanthophoric) pigmentation is present on the dorsal surface of the head and extends ventrolaterally to the eye which appears silvery for the first time. Silvery color (guanine crystals) appears on the preoperculum and operculum posterior to the eye during transformation (13 mm) with lavender pigmentation on the eye and a few orange chromatophores around the opercular spines. During the transformation stage, melanophores rapidly increase in number and spread over all surfaces of the head until the entire head is covered with melanophores obscuring color pigmentation by the beginning of the juvenile stage (20 mm).

Pigmentation: preanal region. Three to five melanophores appear over the dorsal surface of the gut at the beginning of the preflexion stage (2.5 mm). Melanophores over the gut increase in number and fuse to form an internal melanistic shield by 3 mm in preflexion larvae. This shield extends anteroventrally and ventrolaterally with development and is clearly visible until external melanistic pigmentation obscures it early in the juvenile stage. Internal melanophores appear dorsal and ventral to the notochord above the anterior of the gut at 2.6 mm and appear to extend anteriorly to the perineural sheath and hind brain by 5.0 mm. Intemal pigmentation may extend from the notochord nearly to the dorsal margin of the body in 3.6 mm preflexion larvae and flexion larvae. Increasing body musculature and external pigmentation during transformation obscures this pigmentation in later stages. Melanophores appear along the dorsal margin of the body early in the transformation stage. Melanistic pigmentation rapidly increases over the surface of the body during transformation and completely covers the body of small juvenile specimens.

Color first appears on late preflexion larvae (7.4 mm). Yellow pigmentation and silvery color appear along the ventral margin of the melanistic shield lining the peritoneum. Xanthophoric pigmentation spreads anteriodorsally and appears as a wash of color covering the gut region and nape; silvery color covers the ventrolateral region of the peritoneal shield in post-flexion and transformation fish. Color pigmentation in the preanal region becomes obscured by melanistic pigmentation on transformation to the juvenile stage.

Pigmentation: post-anal region. In the post-anal region, melanophores first appear along the ventral midline of the body about one-third post-anal length anterior from the notochord tip at 2.4 mm and increase in number to seven to nine by 2.6 mm. One of the anteriormost melanophores in this series usually appears notably larger than the others. This melanophore appears to be expanded in larvae of 2.8 mm and appears to form what Powell and Tucker (1992) have described as an inverted saddle on the ventrolateral surface of the body in the vicinity of myomeres 17-20. Three to five internal melanophores appear along the dorsal surface of the notochord in the region dorsal to the inverted saddle at 6 mm. Late in the flexion stage (7.4 mm), internal and external pigmentation associated with this saddle begins to move dorsally towards the notochord and lateral midline of the body. By the end of the flexion at 9.6 mm, intemal melanophores extend along the dorsal surface of the notochord from the caudal peduncle to midbody and a patch of external pigmentation has moved to the lateral midline of the body on the caudal peduncle.

Color first appears in 9 mm larvae in the post-anal region. A wash of yellow pigmentation surrounds the melanistic pigment patch on the caudal peduncle and a few orange chromatophores appear along the lateral midline near the caudal peduncle patch. This yellow wash of pigmentation persists through the larval and transformation stages. Orange chromatophores increase in number and by the beginning of transformation (12 mm) extend anteriorly along the lateral midline from the caudal peduncle patch to a position under the origin of the soft dorsal fin. Internal pigmentation, color pigmentation and the caudal peduncle patch are obscured by musculature and external pigmentation by the beginning of the juvenile period at 20 mm.

Pigmentation: fins. Fin pigmentation first appears in the caudal fin membrane, ventral to the notochord tip at 2.6 mm. Two to twelve melanophores generally appear as a line of pigment extending away from the ventral tip of the notochord prior to flexion. As the caudal fin develops, these

melanophores become fewer in number through post-flexion and appear on the fin membrane between the developing caudal rays. During transformation, a few orange chromatophores and a "crescent" area in the anterior third of the fin becomes covered with leucocytes. Towards the end of transformation stage small melanophores appear, except for a crescent-shaped area in the anterior third of the fin, which remains lightly pigmented and appears white due to presence of leucocytes. Melanophores appear flag-like on the fin membrane at the distal tips of the second dorsal and the pelvic fin spines immediately after they begin to develop at 2.9 mm. Melanistic pigmentation over the second dorsal fin spine extends proximally and covers the distal one-third of the spines and is the only melanistic pigmentation present on the spinous and soft dorsal fin until melanistic pigmentation rapidly develops and obscures larval pigmentation during transformation.

Orange pigmentation appears around the flag only during the transformation stage at 12 mm. The spinous dorsal fin membrane of juveniles is densely covered by small melanophores except for the distal fringes between the spines, which lack melanophores and appear as white "flags". Melanistic pigmentation extends proximally over the pelvic fin spine to the pelvic fin base by 5 mm. Melanophores appear on the pelvic fin membrane coinciding with initiation of pelvic fin development (6.5 mm), and persist through larval development. The pelvic fin spine is covered by an orange wash of chromatophores during the post-flexion stage (9.5 mm). Orange chromatophores extend posteriorly onto the pelvic fin membrane and cover the entire pelvic membrane during the transformation stage. Rapid development of melanistic pigmentation on the pelvic fin late in transformation obscures all larval pigmentation in juveniles.

Orange chromatophores appear over the distal tip of the second anal fin spine early in the transformation stage (12.5 mm). By 14 mm, a row of orange chromatophores appear at the insertion of the anal rays and a crescent-shaped band of leucocytes extends across the middle of the anal fin. Larval pigmentation is obscured by melanistic pigmentation by the beginning of the juvenile stage (20 mm), except for the crescent-shaped band of white pigmentation.

Juveniles in rearing aquariums did not quickly acquire the dark juvenile color pattern, which is similar to the adult. Rather, the barred pattern found on large juvenile and adults was first faintly acquired.

Swim bladder. Swim bladder inflation was first noted at day 12 when the silvery-sheen of the swim bladder was noted inside the gut of the larger larvae in the rearing tank.

Larval growth rate. Data for growth rate (Fig. 11) were taken from preserved specimens which show shrinkage averaging 7% after preservation and are indicative of the growth rate of the more advanced larvae in the rearing tanks.

Juvenile growth. Six red grouper juveniles were individually maintained in 80-l aquaria and fed to satiation with juvenile pinfish (*Lagodon rhomboides*). From days 66 to 134, these juveniles grew at rates from 0.26 to 0.61 mm·day^{-1} (mean = 0.41 mm·day^{-1}) (Fig 12). From 134 to 557 days, growth rates were 0.20 to 0.29 mm·day^{-1} (mean = 0.27). All six juveniles were released in October 1992 on an artificial reef offshore of Cedar Key, Florida, in water 15 m deep. They were freeze-branded (Colin and Koenig, in press) and dart-tagged and their survival is being monitored.

Discussion

The eggs of red grouper would be indistinguishable, in the field, from the eggs of most other species of *Epinephelus*. The

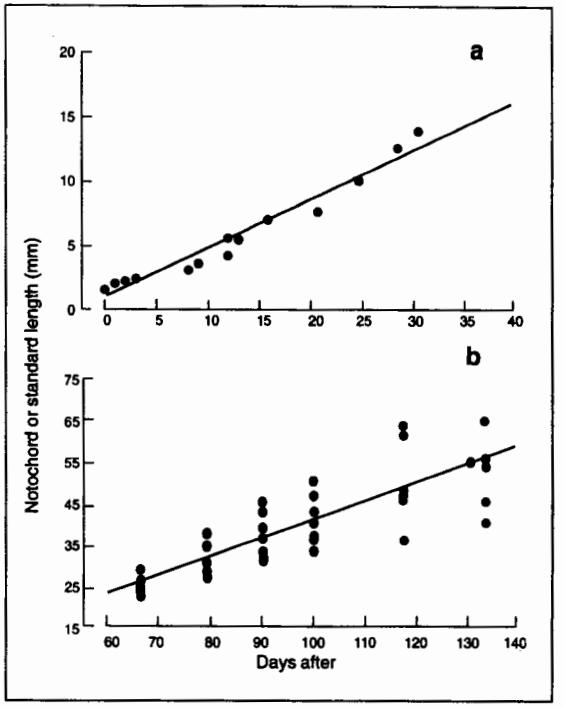

**Fig 11. a) Growth of larval and pelagic juvenile *Epinephelus morio* in the laboratory. [*Crecimiento de larvas y juveniles pelágicos de* Epinephelus morio *en el laboratorio.*] b) Growth of benthic juvenile *Epinephelus morio* in the laboratory. [*Crecimiento de juveniles bentónico de* Epinephelus

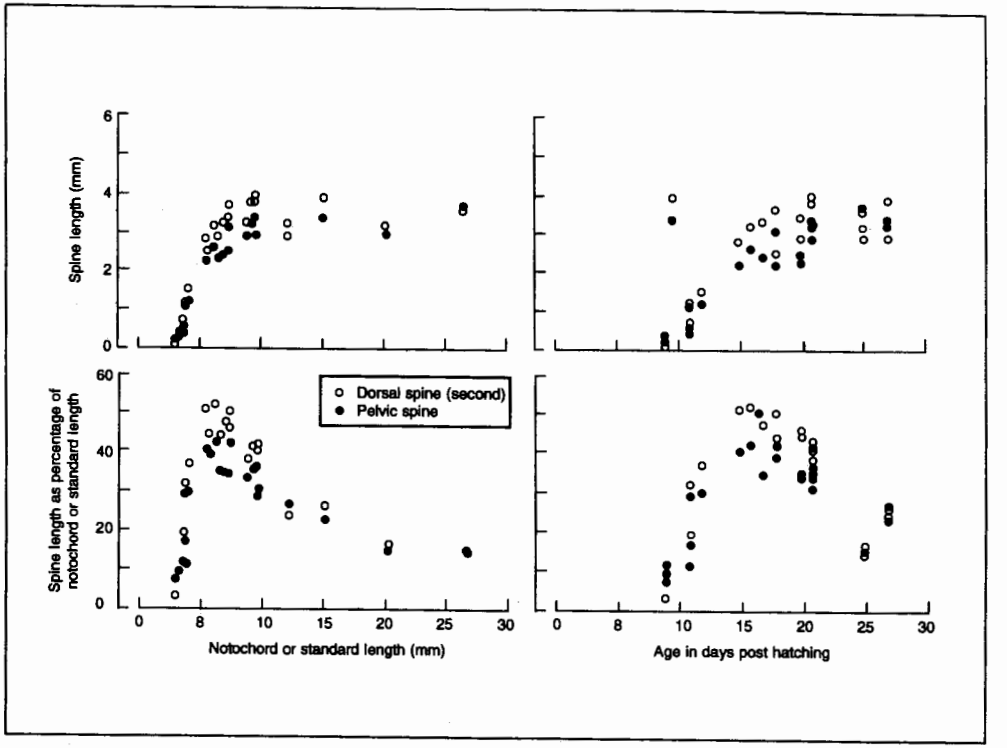

**Fig 12. The relationship of the absolute and relative lengths of the second dorsal and pelvic fin spines of larval *Epinephelus morio* with the length (notochord length in preflexion larvae and standard length in postflexion larvae) and post-hatch age in days. [*Relación entre las longitudes absoluta y relativa de la segunda espina de la aleta dorsal y la espina de la aleta pélvica de larvas de* Epinephelus morio *con la longitud y edad post-crianza, en días (longitud del notocordio en larvas en estado de preflexión, y longitud estándar en larvas en estado de postflexión).*]

density of eggs indicates that they must be spawned intially in water of 28 ppt or greater to be positively buoyant and late stage eggs require about 32 ppt for neutral buoyancy. Almost certainly, eggs which are not floating have little chance of successful development, although embryonic development can proceed. Such eggs would come into contact with the bottom fairly soon after spawning and the conditions would not favor survival of the egg or early larvae.

As salinities lower than the 32 ppt which are necessary for positive buoyancy are common in inshore areas of the coast of Florida of the Gulf of Mexico, this is one factor which would limit the areas where *E. morio* could spawn. There are also often haloclines occurring in this area, and it is probable that eggs would rise to the upper limit of high salinity water and remain there, probably developing successfully.

Spine development

Relative to standard length, the second dorsal fin spine reached its maximum of about 50% and the pelvic spine its peak of 40% of SL at 5.5-7.4 mm SL (Fig. 12). The longest absolute spine lengths were obtained at these same stages. Powell and Tucker (1992) reported slightly smaller relative spines lengths (maxima of 48% and 32%) for laboratory-reared *E. striatus*. For larvae smaller than 6.0 mm SL, they did not report any relative dorsal spine lengths over about 36% and pelvic spine lengths over 27%. For *E. morio*, larvae as small as 4.0 mm SL exceeded these relative values for spine length.

Colin and Koenig (in press) argued that the major function of the elongate dorsal and pelvic spines of planktonic epinephelines was antipredator. The early and rapid development of spines, plus their orientation, resulted in a larvae representing a considerably larger and more difficult prey item to a potential predator. The melanophore-rich fleshy tabs at the tips of the spines were very apparent in live larvae and are thought to mark clearly to potential predators the tips of the spines and the effective size of the larvae. When seen in living larvae the location of the caudal pigment spot tends to enhance the pattern produced by the three outward projecting spines with their dark tips. It is seen as a fourth dark spot centered within the triangle produced by the spine tips.

Larval growth rate

We feel that the growth rates of larvae and pelagic juveniles, shown in Figs. 11 and 12 are relatively similar to those which would occur in nature. Given the rates shown and the timing observed in our rearing aquaria where transformation from pelagic juvenile to benthic juveniles occurred at about 20 mm SL and 35-50 days post-hatch, this agrees reasonably well with ages determined for recruiting grouper juveniles in the field. Unfortunately there are no such data available for *E. morio*, but pretransition ages determined from otolith increments were found to be 37-45 days (mean 41.6) for *E. striatus* and 34-42 days (mean 38.6) for *E. fulvus* in the Bahamas (Colin et al., in press). For other Western Atlantic groupers, *Mycteroperca microlepis* had mean pretransition ages of approximately 42 days and SL of 15 mm and *M. bonaci* of 41 days (Keener et al. 1988). The data presented by Powell and Tucker (1992) for laboratory reared *E. striatus* indicate a slower growth rate for that species. For example, their 17-20 days post-hatch larvae were 5.5-6.5 mm SL, while the present *E. morio* were 5.7- 7.4 mm SL; and at 26-30 days lengths were 8.5-9.4 mm SL vs. 12.1-15.1 mm SL, respectively. Their largest larvae at 13.2 mm SL was of an age (40 days) at which transition of *E. striatus* to benthic existence would be expected, normally at 20 mm

SL or more (Colin et al., in press). Whether their slower larval growth rate was due to rearing conditions needs to be determined.

Keener et al. (1988) also collected a small number of recruiting *E. morio* in nets anchored in a channel leading to an estuarine area off Charleston, South Carolina. Compared to the gag, *Mycteroperca microlepis* and other grouper pelagic juveniles, these comprised less than 1% of the epinepheline catch, but were generally collected during mid-April to mid-May, at a time consistent with the known spawning season of *E. morio* and the time believed required for development to the benthic juvenile stage.

Juvenile growth

The growth rates of the juveniles in aquaria indicate considerable individual variation. However, the mean rate observed, 0.41 mm·day^{-1} is similar to that reported by Colin et al. (in press) for *Epinephelus striatus* in the Bahamas.

The release of the laboratory-reared juveniles on a Florida artificial reef represents, we believe, the first release of cultured groupers into the wild.

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